Very Low Density Lipoprotein

REMOVAL OF APOLIPOPROTEINS C-II AND C-III-1 DURING LIPOLYSIS IN VITRO*

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In this study we have investigated the effects of very low density lipoprotein (VLDL) lipolysis on the removal of radiolabeled apolipoprotein C-II and apolipoprotein C-III-1 from in vitro lipolysed lipoproteins. Lipolysis was carried out *in vitro* using lipoprotein lipase purified from bovine milk, and mixtures with or without plasma. Lipoproteins were isolated by ultracentrifugation and by gel filtration. Labeled apo-C-II and apo-C-III-1 distributed among plasma lipoproteins, predominantly VLDL and high density lipoprotein (HDL). Lipolysis induced transfer of apo-C-II and apo-C-III-1 from VLDL to HDL. The transfer was proportional to the extent of triglyceride hydrolysis, and similar for the two apoproteins. The apo-C-II/apo-C-III-1 radioactivity ratio did not change in either VLDL or the fraction of d > 1.006 g/ml during the progression of the lipolytic process. Similar observations were recorded while using plasma-devoid lipolytic systems. Gel filtration of incubation mixtures, on 6% agarose, revealed that the removal of labeled apo-C molecules from VLDL is not a consequence of either centrifugation or high salt concentration. These results suggest that there is no preferential removal of apo-C-II or apo-C-III-1 from lipolyzed VLDL particles. They further indicate that the ratio of apo-C-II to apo-C-III-1 does not regulate the extent of lipolysis of different VLDL particles, at least in VLDL isolated from normolipidemic humans.

Apolipoprotein C (apo-C-I, apo-C-II, and apo-C-III)§ is part of the protein moiety of plasma lipoproteins (1, 2). These apoproteins contribute 40 to 80% to total protein of chylo- lipoproteins and VLDL are present in plasma HDL (3), and play an important role in the regulation of the activity of the enzyme system lipoprotein lipase (4, 5). In fasting human plasma, apo-C molecules are evenly distributed between VLDL and HDL (3). Recently, it has been demonstrated that the distribution of apo-C molecules between chylomicrons, VLDL, and HDL reflects a dynamic equilibrium (6, 7). APO-C molecules are transferred from VLDL to HDL following abrupt triglyceride hydrolysis, and return to VLDL when newly secreted particles enter the circulation (6). Similar observations were reported during clearance and induction of alimentary chylomicronemia (7). More recently, similar observations were reported *in vitro* and a variety of experimental procedures (8-11). Thus, a relationship between the extent of VLDL triglyceride hydrolysis and the content of apo-C in the lipoprotein was established (8, 9). Yet, whether different apo-C molecules behave similarly or dissimilarly in lipolytic systems has not been studied systematically (12). In view of the reported opposing roles of two of the apo-C species, apo-C-II and apo-C-III, in triglyceride transport (5, 13), we have decided to study in detail their behavior in a lipolytic system containing human plasma VLDL and lipoprotein lipase.

**MATERIALS AND METHODS**

Preparation of Lipoproteins and Apolipoproteins—Human plasma was obtained by plasmapheresis in anticoagulant citrate dextrose solution from normolipidemic humans during morning hours and after a 14-h fast. VLDL was separated at plasma density (1.006 g/ml) by ultracentrifugation in a Beckman 52.1 rotor and a Beckman ultracentrifuge following 18 h spin at 45,000 rpm. The VLDL was washed and concentrated by one additional spin in a Beckman 40 swinging bucket rotor at 40,000 rpm for 18 h. LDL (d = 1.006 to 1.063 g/ml), HDL (d = 1.063 to 1.21 g/ml), and the plasma protein fraction of d > 1.21 g/ml were separated after removal of VLDL from the plasma 1.006 g/ml infranatant by successive ultracentrifugations at densities of 1.063 g/ml and 1.21 g/ml, respectively. Density was adjusted with KBr and KBr solutions of known density. To prepare VLDL density subfractions, the procedure of Lindgren et al. (14, 15) was followed. The efficiency of separation of the VLDL density subfractions was monitored by ultracentrifugation in a zonal rotor as described by Patsch et al. (18). An almost complete separation of the fractions was achieved, as judged from the density profiles.

Apolipoprotein C-II and apolipoprotein C-III-1 were prepared from delipidated human plasma VLDL using gel filtration and ion exchange chromatography on DEAE-cellulose as previously described (17, 18). The purity of the apo-C preparations was monitored by isoelectric focusing on polyacrylamide gels (19). The purity of each apoprotein was greater than 95%.

Preparation of Labeled Apolipoproteins—Apolipoproteins C-II and C-III-1 were labeled with either 131I or 125I by the iodine monochloride method (20) as modified for labeling of lipoproteins (12). The degree of iodination of the apoproteins was kept below 0.5 mol of iodine/mol of protein. Excess radiiodine was removed by dialysis against 0.9% NaCl, 0.01 M Tris (pH = 7.4) buffer. After dialysis, more than 95% of
the remaining radioactivity was precipitated by 10% trichloroacetic acid. The biological nativity of the labeled apolipoproteins was monitored by their capacity to reassociate with lipoproteins. About 95% of the labeled apo-C-II and about 80% of the labeled apo-C-III-I reassociated with lipoproteins when mixed with human plasma (Table I). Recentrifugation of VLDL labeled with the radiodinated apolipoproteins resulted in a minimal (less than 5%) displacement of the apoproteins from the lipoproteins. Only those apo-C preparations that fulfilled the above-described criteria were used in the study.

Na<sup>125</sup>I and Na<sup>131</sup>I were obtained from New England Nuclear. Radioactivity was determined in a Packard Autogamma Spectrometer.

**Analytical Procedures**—Lipoprotein-protein was determined by the Lowry procedure (21). Phospholipids were determined according to the method of Bartlett (22), cholesterol by an enzymatic method (23), and triglycerides by the Autoanalyzer technique (24). In some experiments (see below), lipolysis was carried out in the absence of plasma. After the incubation, the VLDL and postlipolysis VLDL were separated from the albumin and other lipolytic products by gel filtration on Bio-Gel A-5M (a 6% agarose preparation, Bio-Rad). The column, 60 x 0.9 cm, was packed with Bio-Gel A-5M and eluted in 1.0-M fractions with 0.9% NaCl, 0.01 M Tris (pH = 7.4) buffer, and the column fractions were taken for radioactivity determinations. Lipoprotein lipase was purified from bovine milk by affinity chromatography on Sepharose- heparin as previously described (25). The enzyme was prepared in Umeå, Sweden, and was shipped in dry ice to Houston. A typical enzyme preparation contained 350 to 400 units of activity/mg of protein, when tested against triglyceride substrate (26). Loss of activity during shipment and storage at -30°C was negligible.

**Experimental Procedures**—The *in vitro* incubation system consisted of 4 ml of fresh plasma, 1 ml of 20% bovine albumin (Fraction V powder, Sigma), in 0.9% NaCl and aliquots of radiolabeled apo-C-II and apo-C-III-I. The apoproteins were labeled each with either <sup>125</sup>I or <sup>131</sup>I, and the label was reversed in different experiments. The plasma, albumin, and labeled apo-C-II and apo-C-III-I were incubated without enzyme at 37°C in a thermostated water bath for 30 min. Aliquots of lipoprotein lipase were then added to the plasma mixture samples and the samples were further incubated for an additional 30 to 60 min. Several control samples (without lipoprotein lipase) were included in each experiment. Incubations were carried out in cellulose nitrate (40.3 rotor) ultracentrifuge tubes, and the tubes were transferred to ice, filled with ice-cold 0.9% NaCl solution, and spun in a prechilled (4°C) 40.3 rotor at 39,000 rpm for 18 h. VLDL (d < 1.006 g/ml) was isolated by the tube slicing technique (27). LDL (d = 1.006 to 1.063 g/ml), HDL (d = 1.063 to 1.21 g/ml), and the plasma protein fraction of d > 1.21 g/ml were then isolated similarly by successive ultracentrifugations at densities of 1.063 g/ml and 1.31 g/ml for 24 and 48 h, respectively. Radioactivity was determined in all four ultracentrifuge samples, and the <sup>125</sup>I emission was corrected for energy absorption as previously described (28). The recovery of radioactivity after centrifugation was complete, and did not vary in samples incubated with or without lipoprotein lipase (Fig. 1).

In some experiments, lipolysis was carried out without plasma. The experimental design was similar to that described above, with the exception that 0.1 M Tris (pH = 8.2) buffer replaced the plasma in the incubation mixture. In these experiments, labeled apo-C-II and apo-C-III-I were first allowed to associate with VLDL by incubation at 37°C, and the labeled VLDL was then separated by ultracentrifugation from unassociated labeled apo-C (9-11). The labeled VLDL was used for the incubation with lipoprotein lipase, and postlipolysis VLDL was isolated either by ultracentrifugation at NaCl solution of d = 1.019 g/ml or by gel filtration on Bio-Gel A-5M.

The degree of VLDL triglyceride hydrolysis was determined by the triglyceride content of VLDL following incubations and ultracentrifugation.

**RESULTS**

The distribution of labeled apo-C-II and apo-C-III-I between plasma lipoproteins and among VLDL density subfractions was determined after incubations without lipoprotein lipase. More than 90% of the labeled apo-C-II and more than 80% of the labeled apo C III-I were isolated after the incubation with lipoproteins (Table I). The distribution of the two labeled apo-C species between VLDL and HDL paralleled the concentration of these lipoproteins in the different plasma samples (Table I). The ratio of radioactive apo-C-II to apo-C-III-I in VLDL and HDL was similar (Table I) and did not vary much among the three VLDL density subfractions (Table II).

The effects of lipolysis on the distribution of labeled apo-C-II and apo-C-III-I among plasma lipoproteins was determined.

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**TABLE I**

**Distribution of radioactive apo-C-II and apo-C-III-I among plasma lipoproteins**

<table>
<thead>
<tr>
<th>Plasma sample</th>
<th>apo-C-II (125I)</th>
<th>apo-C-III-I (131I)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>40.2</td>
<td>6.2</td>
</tr>
<tr>
<td>2</td>
<td>43.2</td>
<td>6.0</td>
</tr>
<tr>
<td>3</td>
<td>21.8</td>
<td>5.3</td>
</tr>
<tr>
<td>4</td>
<td>96.3</td>
<td>5.7</td>
</tr>
</tbody>
</table>

V powder, Sigma, in 0.9% NaCl and aliquots of radiolabeled apo-C-II and apo-C-III-I. The apoproteins were labeled each with either <sup>125</sup>I or <sup>131</sup>I, and the label was reversed in different experiments. The plasma, albumin, and labeled apo-C-II and apo-C-III-I were incubated without enzyme at 37°C in a thermostated water bath for 30 min. Aliquots of lipoprotein lipase were then added to the plasma mixture samples and the samples were further incubated for an additional 30 to 60 min. Several control samples (without lipoprotein lipase) were included in each experiment. Incubations were carried out in cellulose nitrate (40.3 rotor) ultracentrifuge tubes, and the tubes were transferred to ice, filled with ice-cold 0.9% NaCl solution, and spun in a prechilled (4°C) 40.3 rotor at 39,000 rpm for 18 h. VLDL (d < 1.006 g/ml) was isolated by the tube slicing technique (27). LDL (d = 1.006 to 1.063 g/ml), HDL (d = 1.063 to 1.21 g/ml), and the plasma protein fraction of d > 1.21 g/ml were then isolated similarly by successive ultracentrifugations at densities of 1.063 g/ml and 1.31 g/ml for 24 and 48 h, respectively. Radioactivity was determined in all four ultracentrifuge samples, and the <sup>125</sup>I emission was corrected for energy absorption as previously described (28). The recovery of radioactivity after centrifugation was complete, and did not vary in samples incubated with or without lipoprotein lipase (Fig. 1).

In some experiments, lipolysis was carried out without plasma. The experimental design was similar to that described above, with the exception that 0.1 M Tris (pH = 8.2) buffer replaced the plasma in the incubation mixture. In these experiments, labeled apo-C-II and apo-C-III-I were first allowed to associate with VLDL by incubation at 37°C, and the labeled VLDL was then separated by ultracentrifugation from unassociated labeled apo-C (9-11). The labeled VLDL was used for the incubation with lipoprotein lipase, and postlipolysis VLDL was isolated either by ultracentrifugation at NaCl solution of d = 1.019 g/ml or by gel filtration on Bio-Gel A-5M.

The degree of VLDL triglyceride hydrolysis was determined by the triglyceride content of VLDL following incubations and ultracentrifugation.
Removal of apo-C-II and apo-C-III-I from Lipolyzed VLDL

TABLE II

Distribution of radioactive apo-C-II and apo-C-III-I among VLDL subfractions

VLDL isolated from normal human plasma by centrifugation as described under "Materials and Methods" was incubated for 30 min at 37°C with aliquots of [125I] labeled apo-C-II and [131I] labeled apo-C-III-I. After the incubation, three VLDL density subfractions were prepared by ultracentrifugation in NaCl gradient as described by Lindgren et al. (14, 15). The subfractions, designated 1, 2, and 3 in the order of their floatation, were isolated and characterized by chemical and radiochemical methods.

<table>
<thead>
<tr>
<th>Subfraction</th>
<th>Composition</th>
<th>Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TG* CHOL PL Protein [125I]apo-C-II [131I]apo-C-III-I [125I]/[131I] ratio</td>
<td></td>
</tr>
<tr>
<td>VLDL-1</td>
<td>13.0 ± 3.1 1.6 ± 0.2 3.2 ± 0.6 2.0 ± 0.3</td>
<td>6,620 ± 340 22,290 ± 980 0.399 ± 0.012</td>
</tr>
<tr>
<td>VLDL-2</td>
<td>12.1 ± 1.8 1.8 ± 0.4 2.9 ± 0.4 2.1 ± 0.2</td>
<td>9,420 ± 210 22,340 ± 640 0.454 ± 0.008</td>
</tr>
<tr>
<td>VLDL-3</td>
<td>10.8 ± 1.8 1.8 ± 0.3 4.6 ± 0.7 2.9 ± 0.3</td>
<td>13,400 ± 470 31,140 ± 1,260 0.430 ± 0.020</td>
</tr>
</tbody>
</table>

*The abbreviations used are: TG, triglyceride; CHOL, cholesterol; PL, phospholipid.

Fig. 2. Distribution (%) of radio-labeled apo-C-II (A) and apo-C-III-I (B) among lipoproteins after incubation of human plasma with lipoprotein lipase. Labeled apo-C-II and apo-C-III-I were added to 5 ml of a human plasma-albumin mixture, were incubated at 37°C for 30 min without lipoprotein lipase and for another 30 to 60 min after the addition of 5- to 100-μl aliquots of the enzyme. Lipoproteins of d < 1.006 g/ml (VLDL), d = 1.006 to 1.063 g/ml (IDL + LDL), d = 1.063 to 1.21 g/ml (HDL) and the plasma protein fraction of d > 1.21 g/ml were isolated by ultracentrifugation. The amount of radioactivity associated with each lipoprotein was determined as described under "Materials and Methods." The percent hydrolysis of VLDL-triglycerides was determined by the triglyceride content of VLDL (d < 1.006 g/ml) after centrifugation. Results are of 14 incubations without lipoprotein lipase and 21 incubations with lipoprotein lipase. With lipolysis, redistribution of labeled apo-C-II and apo-C-III-I from VLDL to HDL was observed (Fig. 2).
Removal of apo-C-II and apo-C-III-1 from Lipolyzed VLDL

first to demonstrate this phenomenon in plasma of humans after the injection of heparin (29). Subsequent studies have extended the observation to many other in vivo (6, 7, 12, 30, 31) and in vitro (8-12) systems. These studies have established that apo-C molecules distribute from VLDL and chylomicrons to HDL during degradation of the triglyceride-rich lipoproteins and return to chylomicrons and VLDL when newly synthesized lipoproteins enter the plasma. Whether different apo-C molecules behave similarly in the transfer phenomenon,

Fig. 3. Apo-C-II/apo-C-III-1 radioactivity ratio in VLDL (d < 1.006 g/ml) and in the other plasma lipoproteins and proteins (d > 1.006 g/ml) after incubation with lipoprotein lipase. Data are from 11 incubations without lipoprotein lipase and 21 incubations with lipoprotein lipase as described in legend to Fig. 2. Apo-C-II/apo-C-III-1 radioactivity ratio in VLDL isolated after incubation without lipoprotein lipase was normalized to 1.0; all other ratios are compared to this normalized value.

There was almost no effect of lipolysis on apo C radioactivity in either LDL or the plasma protein fraction of d > 1.21 g/ml. The ratio of radioactive apo-C-II to apo-C-III-1 was determined after centrifugation in VLDL (d < 1.006 g/ml), and the fraction of d > 1.006 g/ml containing all other plasma proteins and lipoproteins. This ratio did not change much throughout the range of VLDL lipolysis (Fig. 3).

Removal of labeled apo-C-II and apo-C-III-1 from VLDL was also observed when lipolysis was carried out without plasma (Fig. 4). VLDL and post-lipolysis VLDL were isolated at d < 1.019 g/ml. Centrifugation of the d > 1.019 g/ml of infranatant at d = 1.21 g/ml (data not shown) revealed that two-thirds to three-quarters of the labeled apo-C-II and apo-C-III-1 removed from VLDL, were isolated with the fraction of d > 1.21 g/ml. During lipolysis, the apo-C-II/apo-C-III-1 radioactivity ratio increased slightly in VLDL and decreased in the buffer infranatant of d > 1.019 g/ml (Fig. 5). These changes were observed only with the initiation of lipolysis (0 to 21.3%) and did not progress with the progression of lipolysis (21.3 to 48.7%). To determine whether removal of labeled apo-C-II and apo-C-III-1 from VLDL may reflect effects of ultracentrifugation, or high salt concentration, or both, samples obtained in this experiment were also separated by gel filtration on Bio-Gel A-5M. The results of the gel filtration experiment (Fig. 6) demonstrated displacement of labeled apo-C-II and apo-C-III-1 from VLDL irrespectively of the isolation method.

DISCUSSION

Redistribution of apo-C molecules between chylomicrons, VLDL, and HDL has been observed consistently whenever a change occurred between the mass ratio of the triglyceriderich lipoproteins to HDL. La Rosa and his associates were the

Fig. 4. Radioactive apo-C-II and radioactive apo-C-III-1 in VLDL (d < 1.019 g/ml) after incubation of human plasma VLDL with lipoprotein lipase in the absence of plasma. VLDL was prepared by ultracentrifugation, incubated with labeled apo-C-II and apo-C-III-1 for 30 min at 37°C, and was separated from unbound apo-C-II and apo-C-III-1 by one additional centrifugation. The labeled VLDL (1 mg of protein) was suspended in 5-ml aliquots of 0.1 M Tris buffer (pH = 8.2) containing 4 g/ml (w/v) bovine serum albumin without and with lipoprotein lipase at 37°C for 60 min. After the incubation, concentrated NaCl solution was added to the mixture in quantities sufficient to raise the density to 1.019 g/ml. VLDL (d < 1.019 g/ml) was isolated by centrifugation, and the amount of radioactive apo-C-II and apo-C-III-1 in the VLDL and in the incubation fraction of d > 1.019 g/ml was determined as described under "Materials and Methods." After incubation without enzyme, 80 to 90% of the labeled apo-C-II and 70 to 80% of the labeled apo-C-III-1 were isolated with VLDL at d < 1.019 g/ml. The percent hydrolysis of VLDL-triglycerides was calculated from the triglyceride content of VLDL (d < 1.019 g/ml) after the incubation. Data are from four incubations without enzyme and nine incubations with enzyme.
system (5); apo-C-III is probably an inhibitor of the enzyme hydrolysis in these lipoproteins has been suggested by several investigators. The possibility that the ratio of apo-C-II to apo-C-III in the plasma, both apoproteins distributed from VLDL to HDL and this transfer reaction progressed in parallel to the degree of triglyceride hydrolysis. We found that 80 to 95% of the labeled apo-C-II and apo-C-III-1 added to human plasma were isolated with lipoproteins, predominantly VLDL and HDL. Following addition of purified lipoprotein lipase to the labeled plasma, both apoproteins distributed from VLDL to HDL and this transfer reaction progressed in parallel to the degree of VLDL triglyceride hydrolysis. We, moreover, found that the disappearance of labeled apo-C-II from VLDL and its appearance in HDL, was very similar to that of apo-C-III-1 and the ratio between the two labeled apoproteins in VLDL and HDL did not change much during lipolysis.

Apo-C-II is a documented activator of the lipoprotein lipase system (5); apo-C-III is probably an inhibitor of the enzyme (13). The possibility that the ratio of apo-C-II to apo-C-III in chylomicrons and VLDL may regulate the rate of triglyceride hydrolysis in these lipoproteins has been suggested by several investigators. Two studies have indicated that the mass ratio of apo-C-II to apo-C-III-1 is decreasing with the decrease in density of VLDL particles, suggesting that the ratio of apo-C-II to apo-C-III-1 may regulate the rate of triglyceride hydrolysis in different particles (36, 37). In another study, however, the ratio of apo-C-II to apo-C-III was similar in VLDL subfractions prepared by either ultracentrifugation or gel filtration (38). More recently, Catapano et al. have restudied this problem using disc-gel isoelectric focusing techniques (19). A similar mass ratio between C apoproteins in different VLDL density fractions was found. In the present study we observed a similar ratio of labeled apo-C-II to apo-C-III-1 in VLDL density fractions. During lipolysis, moreover, when VLDL particles became denser and smaller (7, 15), this ratio has not changed much. These observations suggest that the ratio of apo-C-II to apo-C-III does not regulate triglyceride hydrolysis in VLDL particles, at least in normal human plasma. It is interesting to note in this context that restoration of 10 to 20% of the apo-C-II pool in the plasma of a patient with apo-C-II deficiency by means of plasma infusion is sufficient to cause pronounced lipolysis of chylomicrons and VLDL (39). After the plasma infusion, the apo-C-II to apo-C-III mass ratio in the patient’s plasma is probably a small fraction of that of normal humans.

What determines the distribution of apo-C molecules between lipoproteins is not clear. VLDL and HDL contain relatively large quantities of apo-C molecules whereas only minimal amounts are found in LDL. This distribution does not reflect any known characteristic of the lipoproteins, i.e. lipid composition, apoprotein profile, size, and radius of curvature or lipid/protein ratios. We suggest that chemical, or physical features of the lipoproteins’ outer shell, or both, as yet undefined, are responsible for the “binding” of apo-C to any given lipoprotein. This “binding property” is best defined by association and dissociation constants that vary from one to the other.

**Fig. 5.** Apo-C-II/apo-C-III-1 radioactivity ratio in VLDL (d < 1.019 g/ml) and the buffer fraction of d > 1.019 g/ml after incubation of doubly labeled VLDL with lipoprotein lipase in the absence of plasma. Data are from the four incubations without lipoprotein lipase and the nine incubations with lipase described in Fig. 4. Apo-C-II/apo-C-III-1 radioactivity ratio in VLDL isolated after incubation without lipoprotein lipase was normalized to 1.0; all other ratios are compared to this normalized ratio.

However, has been unclear. This uncertainty is due in part to methodological difficulties in separating apo-C molecules. In the present study we have attempted to overcome this difficulty by incorporating apo-C-II and apo-C-III-1, labeled differentially with two iodine isotopes, into VLDL and other plasma lipoproteins. Similar methods were used recently to label lipoproteins with apo-A-I (12, 32-34), apo-A-II (12, 33, 34), apo-C of rat plasma lipoproteins (35), and apo-C-II, apo-C-III-1, and apo-C-III-2 of human plasma VLDL origin (12). In the present study we found that 80 to 95% of the labeled apo-C-II and apo-C-III-1 added to human plasma were isolated with lipoproteins, predominantly VLDL and HDL. Following addition of purified lipoprotein lipase to the labeled plasma, both apoproteins distributed from VLDL to HDL and this transfer reaction progressed in parallel to the degree of VLDL triglyceride hydrolysis. We, moreover, found that the disappearance of labeled apo-C-II from VLDL and its appearance in HDL, was very similar to that of apo-C-III-1 and the ratio between the two labeled apoproteins in VLDL and HDL did not change much during lipolysis.

**Fig. 6.** Elution profile on Bio-Gel A-5M of [125I]apo-C-II and [131I]apo-C-III-1 of labeled VLDL after incubation without and with lipoprotein lipase. Incubations of doubly labeled VLDL were carried out in 0.1 M Tris, 4% albumin buffer as described in legend to Fig. 4. At the end of 60-min incubation, 0.5-ml aliquots of the incubation mixture were applied to a Bio-Gel A-5M column (60 x 0.9 cm). The column was eluted with 0.9% NaCl, 0.01 M Tris buffer, pH = 7.4, at 1.0-ml fractions. Radioactivity content was then assessed as described under "Materials and Methods." In the experiment shown here, the per cent hydrolysis of VLDL-triglyceride was 52.1%.
lipoprotein to another, and reflects the properties of the outer coat of the lipoprotein (40). At equilibrium, the distribution of apo-C between the lipoproteins is best explained as taking place through the water phase, as suggested recently (40, 41). The redistribution of apo-C between VLDL and HDL during lipolysis, according to the hypothesis, reflects the changing mass ratio between the two, as well as possible changes in the properties of the lipoproteins. These changes are caused by deletion of core and surface constituents from VLDL (10, 11); HDL is modified—simultaneously, being an acceptor of cholesterol and phospholipids freed from the lipolyzed VLDL (42). Removal of apo-C-II and apo-C-III-I from Lipolyzed VLDL.

REFERENCES

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